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We propose that the expression of several unknown genes is affected by gamma radiation. Abnormal expression of these genes may be one of the early steps in breast carcinogenesis induced by radiation. We plan to establish an assay that will allow us to screen for breast cells that contain a single mutation by gene trapping. We will be able to detect changes in the expression of a gene upon treatment with different doses of radiation. These radiation-responsive genes will be identified through the rapid amplification of cDNA ends procedure and sequenced. Cells that are affected by radiation will be isolated and analyzed to see if the changes can lead to transformation of the normal breast epithelial cell into a neoplastic cell. This assay may prove to be a powerful tool in the identification of novel genes that are affected by gamma irradiation in the early stages of breast cancer progression.

The 3'RACE protocol has recently been completed and thirty-one potential genes were sequenced. Of these, six candidate genes were found. They include: human creatine kinase gene, human androgen receptor, human DORA reverse strand protein 1 (DREV1), human eukaryotic translation elongation factor 1 beta 2 (EEF1B2), human ribosomal protein L27, and human DNA clone RP11-290F20 on chromosome 20. These genes will be further analyzed for their transformation properties of human mammary epithelial cells as discussed in the statement of work as well as their response to ionizing radiation.

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INTRODUCTION

We propose that the expression of several unknown genes is affected by gamma irradiation. The subject and purpose of our research is that the abnormal expression of these genes may be one of the early steps in breast carcinogenesis induced by radiation. We plan to establish an assay that will allow us to screen for breast cells that contain a single mutation by gene trapping. We will be able to detect changes in the expression of a gene upon treatment with different doses of radiation. These radiation-responsive genes will be identified and analyzed to see if the changes can lead to transformation of the normal breast epithelial cell into a neoplastic cell. This assay may prove to be a powerful tool in the identification of novel genes that are affected by gamma irradiation in the early stages of breast cancer progression.

BODY

RESEARCH TRAINING

Ongoing training is very important throughout my predoctoral period. My department, Environmental and Radiological Health Sciences, places an important focus on training. Weekly, I attend Advanced Radiation Biology scientific journal meetings where faculty and students interact and discuss current and relevant papers in breast cancer research and radiation effects. Each attendee presents one journal article every semester and leads the discussion. Weekly there is a Cell and Molecular Biology seminar where invited visiting speakers give a 50 minute presentation and discussion about relevant topics such as breast cancer research, cell signaling, and many more. There are also two departmental seminars I attend weekly where visiting speakers, as well as graduate students present their research. It is very important for my training that I keep current with the latest research techniques and discoveries by attending these meetings. My mentor, Dr. Robert Ullrich, is currently the Oncology Chair of the Veterinary Teaching Hospital here on campus, so I am also exposed to more clinical cancer research seminars and meetings that I attend there as well.

In October 2003 I attended the American Association for Cancer Research special conference on the Advances in Breast Cancer Research meeting in Huntington Beach, California. In February 2004 I attended the American Association for Cancer Research special conference on Radiation Biology and Cancer meeting in Dana Point, California. In April 2004 I attended the Radiation Research Society Annual meeting in St. Louis, Missouri. These meetings contributed a great deal to my overall predoctoral training by exposing me to breast cancer research scientists from all over the world. I was able to attend numerous oral and poster presentations and learn about the latest advances being made in breast cancer and radiation research.

RESEARCH PURPOSE & GOALS

We plan to identify novel genes affected by gamma irradiation and to characterize their function using a gene-trapped library of human mammary epithelial cells. We hypothesize that the mutation of these novel genes or their abnormal expression is one of the causes of early breast carcinogenesis. Mounting evidence suggests that gene products may function differently depending on cell type, developmental stage, or species. Thus, to identify novel gene(s) critical for the initiation of breast cancer, we need to study the irradiation effects of "loss of function" of a gene product in human breast epithelial cells.

The issue of how low dose gamma radiation may lead to breast cancer will be addressed by studying the genes affected by low dose gamma irradiation. We will focus on the trapped genes whose expression are immediately changed by a single dose of gamma irradiation, determine if this is a dose-dependent effect and further analyze whether this effect can lead to transformation of the breast cells.

The following are the specific aims as outlined in the approved statement of work:

Specific Aim 1: To establish a high throughput assay for detection of variation in gene expression in human mammary epithelial cells using gene-trapped MCF10A clones.
Specific Aim 2: To determine the effect of gamma irradiation on expression of reporter protein GFP (green fluorescent protein).
Specific Aim 3: To characterize the effect of gamma irradiation on transformation of human mammary epithelial cells.
Specific Aim 4: To identify the trapped genes affected by gamma irradiation.

RESEARCH PROGRESS

Currently, specific aim 1, specific aim 2, specific aim 3 and specific aim 4 are completed. Attached in the appendices are color representations of the completed construction of the gene-trapped MCF10A clonal library as seen under a fluorescent microscope. This is included in one of my PowerPoint presentations. It is clearly observed in the pictorials, that the bright green fluorescence luminating from the cells is due to the retrovirus pRET being incorporated into the genome.

A total of 192 gene-trapped clones were analyzed by the construction of a single cell assay in 96-well plates. This was done to obtain single cell clones, hopefully each representing a different trapped gene. One 96-well plate contained the pooled gene-trapped MCF10A cells sorted by flow cytometry into a GFP positive pool and the other 96-well plate contained the pooled gene-trapped MCF10A cells sorted by flow cytometry into a GFP negative pool. Graphical representations of the flow cytometry data are included in one of my power point presentations. Replica plating was then done from both of the original single cell assay plates for the following GFP expression levels to be measured at: basal, control, master, store at -80°C, 0.5 Gy, and 2.0 Gy gamma irradiated. GFP measurements were made with a microplate reader by the way of a sandwich ELISA assay. The sandwich ELISA assay was accomplished by first expanding the 96-well plates with the single cell clones into 24-well plates. These 24-well plates were then expanded further to allow for 2 wells for each single cell assay clone. This was done so that one well could be further expanded and frozen for later use and the other well would be utilized to collect the cell lysate from for the ELISA assay. All of

the 24-well plates were then irradiated with 2.0 Gy from a ^{137}Cs source. The following antibodies were used for the sandwich ELISA assay: anti-GFP (Mouse) was the primary antibody and peroxidase IgG mouse (Rabbit) was the secondary antibody.

Graphical representations of the gene expression of GFP after 2.0 Gy gamma radiation dose from a ^{137}Cs source is attached as well. Here, clones that were up- or down-regulated at least 2-fold from basal readings were expanded for further analysis. The basal GFP readings of the gene-trapped clones are included in the appendices for comparison to the 2.0 Gy GFP readings. Out of the 192 clones analyzed, 92 were up- or down-regulated at least 2-fold in comparison to basal GFP readings. These clones were expanded in culture and RNA was collected for gene analysis.

There was a slight change in the order of the approved statement of work next. Specific aim 4 was undertaken prior to the characterization of the effect of gamma irradiation on the transformation of the MCF10A cells. This was felt to be an important substitution due to the fact that the gene that had been trapped should be identified before transformation assays were undertaken. Transformation assays are very tedious and time consuming. If, for example, the gene trapped was an artifact, then the process of analyzing for transformation could be skipped.

Specific aim 4 dealt with the characterization of the trapped genes that were causing either an up- or down-regulation upon treatment with 2.0 Gy. To analyze this, the gene-trapped clones were expanded and RNA was collected by using Qiagen's RNeasy kit. The protocol for this procedure is attached in the appendices. The RNA was then reverse transcribed into cDNA and amplified by the use of the Advantage-GC cDNA polymerase kit from BD Biosciences and the 3'RACE protocol from Invitrogen. Both protocols are included in the appendices. Gene specific primers for the neomycin marker found on our pRET retrovirus and against the polyA tail of the endogenous gene were designed. After each step, reverse transcription, first strand cDNA synthesis, and second strand cDNA synthesis, agarose gels were run to verify that the gene products were of the correct size.

When a gene product was of the correct size they were PCR purified by Qiagen's PCR purification kit and transformed into One Shot competent E. coli cells via a TOPO Cloning kit from Invitrogen. The transformed clones were then added to LB media and grown overnight. Clones were growth had occurred were then subjected to Qiagen's mini prep kit to harvest the DNA. Protocols for both of these procedures can be found in the appendices. The mini prep clones are then subjected to PCR with M13 primers and run on 1.5% agarose gels. Gel electrophoresis images are provided in the appendices in one of my PowerPoint presentations to illustrate which clones were selected to be sequenced.

Sequencing of the positive mini prep clones was completed at Davis Sequencing which is located at the University of California at Davis. A total of 31 clones were sent off for sequencing and six yielded positive results. The six genes were determined by plugging the sequences of my clones into BLAST and searching for homologous genes. The other clones were determined to be artifacts of the cloning vector. The genes that were trapped were: human creatine kinase gene, human DORA reverse strand protein 1 (DREV1), human eukaryotic translation elongation factor 1 beta 2 (EEF1B2), human ribosomal protein L27, and human DNA clone RP11-290F20 on chromosome 20.

The genes identified through sequencing analysis were expanded for RNA collection and analyzed by real-time PCR. This experiment was performed in order to analyze gene expression of the genes identified through trapping in both the gene-trapped clones and in the parental MCF10A cell line with and without ionizing radiation treatment. We felt that it was important to analyze the identified gene expression levels following IR treatment in the parental cell line to verify that in fact we were in deed seeing a radiation response. These expression levels could also then be compared to the breast cancer cell line, MCF7. In the appendices I have included my real-time PCR protocol and the sequences for the primers and probes that were utilized. Real-time PCR experiments were conducted on an Applied Biosystems 7000 Sequence Detection System with the TaqMan Gold RT-PCR Kit. Also, graphical representations of the relative gene expression of the genes of interest in various time course experiments following ionizing radiation (IR) and after varying doses of IR are included. The time course experiments were conducted at 2, 4, 8, 12, 24, and 30 hours post ionizing radiation treatment with a dose of 2.0 Gy. Time course studies were also completed at the above mentioned time points for 0.10, 0.25, 0.5 Gy, 1.0 Gy, and 4.0 Gy. All five genes were found to illustrate a radiation response to each dose at all the mentioned time points and their relative gene expression and n-fold difference in comparison to the parental, MCF10A cell line, were analyzed. The experimental results from the above mentioned items are all included in the appendices.

RNA interference was targeted to the novel, DREV1 gene in order to determine if MCF10A cell survival was affected following radiation treatment. Cell survival was analyzed with chromosomal and chromatid aberration scoring following radiation treatment. Upon analysis, it was determined that the DREV1 gene did not appear to affect cell survival following radiation treatment in the breast epithelial cell line. A total of 50 metaphase spreads were analyzed for the RNAi DREV1 knockout sample, parental MCF10A sample, irradiated MCF10A sample and irradiated RNAi DREV1 knockout sample.

Specific aim 3 focused on whether the identified radiation response genes could induce transformation in the MCF10A human breast epithelial cell line. Upon investigation, it was determined that the five identified radiation response genes did not induce transformation in MCF10A cells. As of now no known documentations have occurred illustrating radiation-induced transformation in the MCF10A cells.

KEY RESEARCH ACCOMPLISHMENTS

- Five radiation response genes were found to be homologous to known genes through a BLAST search. These genes include: human creatine kinase gene, human DORA reverse strand protein 1 (DREV1), human eukaryotic translation elongation factor 1 beta 2, human androgen receptor, human ribosomal protein L27, and human DNA clone RP11-290F20 on chromosome 20.

- Genes of interest were found to response to a 0.10, 0.25, 0.50, 1.0, 2.0 and 4.0 Gy doses of ionizing radiation and time course experiments were completed to find when peak expression levels following IR occurred. This was analyzed by real-time PCR.
- Cell cycle analysis was done to verify that there was not a cell cycle delay or block causing some of the large changes in expression of some of the genes that was seen.
- Real-time PCR analysis was performed to analyze the expression of our genes of interest at 0.10, 0.25 0.5 Gy, 1.0 Gy, 2.0 Gy, and 4.0 Gy doses of IR.
- One of the genes of interest, DREV1, has a small gene called DORA located in intron 4 on the complement strand. Real-time PCR analysis has been completed to investigate if its gene expression is also affected by the IR doses.
- RNA interference was performed on the DREV1 gene to analyze whether its subsequent knockdown in MCF10A cells affected cell survival.
- Chromosomal and chromatid analysis was performed on the DREV1 knockdown MCF10A cells to access cell survival following ionizing radiation treatment.

REPORTABLE OUTCOMES

- Two scientific papers are in preparation at this time.
- I was invited to give a poster presentation at the Radiation Research Society's 51st Annual Meeting. It was held in April 2004 in St. Louis, Missouri. My abstract can be found in the appendices.
- I was invited to give a 50-minute oral presentation on my research to the faculty, staff and students of the Department of Pathology at the University of Colorado Health Sciences Center. The PowerPoint slides from my presentation are given in the appendices.
- On March 18, 2004 I gave a 20-minute oral presentation on my research to the faculty and students of the Department of Radiological and Environmental Health Sciences. The PowerPoint slides from my presentation are given in the appendices.
- I was invited to give a 50-minutes oral presentation on my research to the faculty, staff and students of the Department of Obstetrics & Gynecology at the University of Colorado Health Sciences Center.
- I was invited to give a poster presentation at Colorado State University for the Cell and Molecular Biology Interdisciplinary Graduate Program Graduate Student and Post Doc Poster Competition on February 27, 2004. My poster abstract is found in the appendices.
- I was invited to give a poster presentation at the American Association for Cancer Research Special Conference: Radiation Biology and Cancer. It was held from February 18th through February 22nd, 2004 in Dana Point, California. My abstract can be found in the appendices.
- I was invited to give a poster presentation at the American Association for Cancer Research Special Conference: Advances in Breast Cancer Research. It was held in October 2003 in Huntington Beach, California. My abstract can be found in the appendices.
- On October 23, 2003 I gave a 20-minute oral presentation on my research to the faculty and students of the Department of Radiological and Environmental Health Sciences. The department has doctoral students give oral presentations every semester on how their research is progressing and any new findings. The PowerPoint slides from my presentation can be found in the appendices.
- On April 17, 2003 I gave a 20-minute oral presentation on my research to the faculty and students of the Department of Radiological and Environmental Health Sciences. The department has doctoral students give oral presentations every semester on how their research is progressing and any new findings. The PowerPoint slides from my presentation can be found in the appendices.
- I was invited to give a 20 minute oral talk and a poster presentation at the Minority Trainee Research Forum sponsored by NIH-National Institute of Diabetes and Digestive Kidney Diseases, NIH-National Institute of Allergy and Infectious Diseases, NIH-Office of Research on Women's Health, and Merck and Company. It was held March 14-17, 2003 at the Westgate Hotel in San Diego, California.
- I was invited to give a poster presentation at Colorado State University for the Cell and Molecular Biology Interdisciplinary Graduate Program Graduate Student and Post Doc Poster Competition on February 21, 2003.
- On November 21, 2002 I gave a 20-minute oral presentation on my research to the faculty and students of the Department of Radiological and Environmental Health Sciences.
- The development of a gene-trapped cell library of MCF10A cells was accomplished with the retrovirus pRET.

Appendices

Primer sequences used for 3'RACE & Sequencing:
AD Poly (T): 5'-CGTAGCTCTAGACTCCGTGTCCAACTTTTTTTTTTTTTTTTTTTT-3'
AD (T): 5'-CGTAGCTCTAGACTCCGTGTCCAACT-3'
NEO1.5: 5'-GCGAATGGGCTGACCGCTTCCTCGTGC-3'
AD: 5'-CGTAGCTCTAGACTCCGTGTCCAAC-3'
NEO2.0: 5'-TACGGTATCGCCGCTCCCGATTCGCAG-3'
AD PLUS: 5'-CGTAGCTCTAGACTCCGTGTCCAACTTTT-3'
NEO SEQ: 5'-TGACGAGTTCTTCTGAGGGGATCC-3'
M13 Forward: 5'-GTAAAACGACGGCCAG-3'
M13 Reverse: 5'-CAGGAAACAGCTATGAC-3'

Primer and Probe sequences for real-time PCR:
Androgen F1: 5'-CCCTGGCGGCATGGT-3'
Androgen F2: 5'-ACCCTGGCGGCATGGT-3'
Androgen F3: 5'-TACCCTGGCGGCATGGT-3'
Androgen R1: 5'-CCCATTTCGCTTTTGACACA-3'
Androgen R2: 5'-CCCATTTCGCTTTTGACACAA-3'
Androgen R3: 5'-GCCCATTTCGCTTTTGACA-3'
DORA F1: 5'-GAGGCAGGGTCATCCTTGC-3'
DORA F2: 5'-GAGCCAACTAGAGGCAGGGTC-3'
DORA F3: 5'-GCCAACTAGAGGCAGGGTCA-3'
DORA R1: 5'-CCCACTTGCCACCTACGTTT-3'
DORA R2: 5'-TCCCACTTGCCACCTACGTT-3'
DORA R3: 5'-CTCCCACTTGCCACCTACGT-3'
ANDROGEN PROBE: 6FAM-AGCAGAGTGCCCTATCCCAGTCCA-TAMRA
DORA PROBE: 6FAM-CTTGTCCTCCCCTTTCATCCCTATGTGG-TAMRA
CK F1: 5'-TGCTACCATGGGCACCAGT-3'
CK F2: 5'-TTGCTACCATGGGCACCACT-3'
CK F3: 5'-TTGCTACCATGGGCACCAG-3'
CK R1: 5'-GCACACACTTTCTGCCGGT-3'
CK R2: 5'-GCACACACTTTCTGCCGGTT-3'
CK R3: 5'-GGCACTCGGCCATGCA-3'
L27 F1: 5'-GCCCCTACAGCCATGCTCT-3'
L27 F2: 5'-ATCGCCCTACAGCCATG-3'
L27 F3: 5'-TCAGATCGCCCCTACAGCC-3'
L27 R1: 5'-CATGGCAGCTGTCACTTTGC-3'
L27 R2: 5'-CCCATGGCAGCTGTCACTT-3'
L27 R3: 5'-TCTTGGCGATCTTCTTCTTGC-3'
EEF1B2 F1: 5'-CACAATTTGCGCGCTCTCT-3'
EEF1B2 F2: 5'-CCACAATTTGCGCGCTCT-3'
EEF1B2 F3: 5'-CCACAATTTGCGCGCTC-3'
EEF1B2 R1: 5'-ACCCATGGTGTCGGCTGTA-3'
EEF1B2 R2: 5'-ACCCATGGTGTCGGCTGT-3'
EEF1B2 R3: 5'-AACCCATGGTGTCGGCTGTA-3'
CK PROBE: 6FAM-TCCTGACCACCGGGTACCTGCTG-TAMRA
L27 PROBE: 6FAM-TGGCTGGAATTGACCGCTACCCC-TAMRA
EEF1B2 PROBE: 6FAM-TCTGCTGCTCCCCAGCTCTCGC-TAMRA

For the protocols used from Qiagen, Invitrogen and BD Biosciences, please visit the following websites:
http://www.invitrogen.com/content/sfs/manuals/topotaseq_man.pdf (TOPO TA Cloning)
http://www.invitrogen.com/content/sfs/manuals/18373019.pdf (3'RACE)
http://www1.qiagen.com/literature/handbooks/PDF/DNACleanupAndConcentration/QQ_Spin/1021422_HBQQSpin_072002WW.pdf (PCR purification)
http://www1.qiagen.com/literature/handbooks/PDF/RNAStabilizationAndPurification/FromAnimalAndPlantTissuesBacteriaYeastAndFungi/RNY_Mini/1016272HBRNY_062001WW.pdf (RNeasy Mini Kit)
http://www1.qiagen.com/literature/handbooks/PDF/PlasmidDNAPurification/PLS_QP_Miniprep/1023790_HBQP_Miniprep_0303.pdf (Miniprep kit)
http://www.clontech.com/techinfo/manuals/PDF/PT1580-1.pdf (Advantage-GC cDNA Polymerase kit)

General Protocol for the Sandwich ELISA method:

1. Before the assay, both antibody preparations should be purified and one must be labeled.
2. For most applications, a polyvinylchloride (PVC) microtiter plate is best; however, consult manufacturer guidelines to determine the most appropriate type of plate for protein binding.
3. Bind the unlabeled antibody to the bottom of each well by adding approximately 50 m L of antibody solution to each well (20 m g/mL in PBS). PVC will bind approximately 100 ng/well (300 ng/cm2). The amount of antibody used will depend on the individual assay, but if maximal binding is required, use at least 1 m g/well. this is well above the capacity of the well, but the binding will occur more rapidly, and the binding solution can be saved and used again.
4. Incubate the plate overnight at 4° C to allow complete binding.
5. Wash the wells twice with PBS. A 500 mL squirt bottle is convenient. The antibody solution washes can be removed by flicking the plate over a suitable container.
6. The remaining sites for protein binding on the microtiter plate must be saturated by incubating with blocking buffer. Fill the wells to the top with 3% BSA/PBS with 0.02% sodium azide. Incubate for 2hrs to overnight in a humid atmosphere at room temperature. (Note: Sodium azide is an inhibitor or horseradish peroxidase. Do not include sodium azide in buffers or wash solutions, if an HRP-labeled antibody will be used for detection.)
7. Wash wells twice with PBS.
8. Add 50 m L of the antigen solution to the wells (the antigen solution should be titrated). All dilutions should be done in the blocking buffer (3% BSA/PBS with 0.02% sodium azide). Incubate for at least 2 hrs at room temperature in a humid atmosphere.
9. Wash the plate four times with PBS.
10. Add the labeled second antibody. The amount to be added can be determined in preliminary experiments. For accurate quantitation, the second antibody should be used in excess. All dilutions should be done in the blocking buffer.
11. Incubate for 2 hrs or more at room temperature in a humid atmosphere.
12. Wash with several changes of PBS.
13. Add substrate as indicated by manufacturer. After suggested incubation time has elapsed, optical densities at target wavelengths can be measured on an ELISA reader.

PCR conditions:
First strand cDNA synthesis:
94°C 3 minutes; 94°C 40 seconds, 72°C 4 minutes, 8 cycles; 94°C 40 seconds, 66°C 2 minutes, 72°C 2 minutes, 32 cycles; 72°C 4 minutes, 4°C overnight.
Second strand cDNA synthesis:
94°C 1 minute, 94°C 40 seconds, 72°C 4 minutes, 8 cycles; 94°C 40 seconds, 66°C 2 minutes, 72°C 2 minutes, 32 cycles; 72°C 4 minutes, 4°C overnight.
Mini prep PCR:
94°C 2 minutes; 94°C 1 minute, 55°C 1 minute, 72°C 1 minute, 25 cycles; 72°C 7 minutes.
Real-time PCR:
50°C 2 minutes; 95°C 10 minutes; 95°C 15 seconds, 60°C 1 minute, 60 cycles.

AACR Radiation Biology Abstract

Breast cancer may be induced with relatively high frequency by radiation. Ionizing radiation is one of the main treatment modalities used in the management of cancer. A radiation dose-related increase in the incidence of breast cancer has been seen in women. When a woman receives significant radiation prior to the age of 20, she becomes more likely to develop breast cancer. Thus there is considerable interest in understanding the cellular response to DNA-damaging agents, particularly because the ability to deliver a curative dose of radiation is frequently limited by the adverse reaction of normal tissues within the radiation treatment field. One approach to this problem is to understand the molecular mechanisms underlying the radiation responses of normal tissue so that critical molecular pathways can be manipulated to improve the therapeutic ratio and hence, the chance of a cure. We propose that the expression of several unknown genes is affected by gamma irradiation. The subject and purpose of our research is that the abnormal expression of these genes may be one of the early steps in breast carcinogenesis induced by radiation. We plan to identify novel genes affected by gamma irradiation and to characterize their function using a gene-trapped library of human mammary epithelial cells. We hypothesize that the mutation of these novel genes or its abnormal expression is one of the causes of early breast carcinogenesis. Mounting evidence suggests that gene products may function differently depending on cell type, developmental stage, or species. Thus, to identify novel gene(s) critical for the initiation of breast cancer, we need to study the irradiation effects of "loss of function" gene products in human breast epithelial cells. We will focus on the trapped genes whose expression are immediately changed by a single dose of gamma irradiation, determine if this is a dose-dependent effect and further analyze whether this effect can lead to transformation of the breast cells. We plan to screen breast cells that contain a single gene mutation using a technique called gene trapping. We will be able to detect changes in the expression of a specific gene upon treatment with different doses of radiation. These radiation response genes will be identified through the rapid amplification of cDNA ends (RACE) procedure and sequenced. Cells that are affected by radiation will be isolated and further analyzed to see if the changes can lead to the malignant transformation of the normal breast epithelial cell into a neoplastic cell. In order to verify that a radiation response is being seen, we will be analyzing the expression of the identified trapped genes in the parental MCF10A cell line both before and after radiation treatment. This assay may prove to be a powerful tool in the identification of novel genes that are affected by gamma irradiation in the early stages of breast cancer progression. This study will provide new information on the effects of radiation and genes that can cause breast cancer that are induced by radiation as well as identify markers for early detection of breast cancer and targets for therapeutic intervention.

Novel radiation response genes identified in MCF10A gene-trapped cells.

Jennifer Malone and Robert Ullrich
Department of Environmental and Radiological Health Sciences, Colorado State University

Abstract Category: Mutagenesis/Clastogenesis/Carcinogenesis

Objective/Hypothesis: In this study, we plan to establish an assay to identify novel genes that are affected by gamma irradiation and to characterize their function and role in early breast carcinogenesis. We hypothesize that the mutation of these genes or their abnormal expression in response to gamma irradiation is one of the causes of breast carcinogenesis.

Specific Aims: The specific aims of this study are:

1. To establish a high throughput assay for detection of variation in gene expression in human mammary epithelial cells using gene-trapped MCF10A clones; 2. To determine the effect of gamma irradiation on the expression of the reporter, green fluorescent protein (GFP); 3. To characterize the effect of gamma irradiation on the transformation of human mammary epithelial cells; 4. To identify the trapped genes affected by gamma irradiation in breast epithelial cells.

Methods: We plan to establish an assay that will allow us to screen for breast cells that contain a single gene mutation using a technique called gene trapping. We will be able to detect changes in the expression of a specific gene upon treatment with different doses of radiation. These radiation-responsive genes will be identified through the rapid amplification of cDNA ends (RACE) procedure and sequenced. Gene-trapped clones that are affected by radiation will be isolated and further analyzed to see if the varying radiation doses can lead to malignant transformation.

Results: The MCF10A gene-trapped library has been established. Basal GFP levels have been measured. Gamma irradiation of the single cell clones at both 1.0 and 2.0 Gy has been performed. Clones that were either up- or down-regulated at least 2-fold in response to the radiation treatment have been expanded for RACE and sequencing analysis. The genes identified through sequencing have been analyzed by real time PCR.

Study Design: Using the poly-A trap retrovirus RET, we have established a gene-trapped library of clones from human mammary epithelial cells (MCF10A). It provides the strong base for the identification of novel genes that may be involved in essential signaling pathways in human mammary epithelial cells. We propose to establish a detection assay using the reporter gene GFP that has been incorporated into the genome of the cells, whose expression is regulated by endogenous promoters of the trapped genes. We will compare basal GFP expression before and after exposure to varying low doses of gamma radiation (0-2 Gy) using replica plates of MCF10A gene-trapped clones. We will then identify the gene(s) involved by using a polymerase chain reaction protocol and sequencing analysis. Next, we will further characterize the clones that are affected by gamma irradiation by performing colony formation assays (to determine survival), anchorage-independent growth and tumorigenicity assays on transformed clones that grow in soft agar.

Conclusions: This assay may prove to be a powerful tool in the identification of novel genes that are affected by gamma irradiation in the early stages of breast cancer progression. This study will provide new information on the effects of radiation-responsive genes that can lead to breast cancer as well as identify new markers for early detection of breast cancer. This study will focus on the identification of novel genes that are potential targets of gamma irradiation. It will provide essential information on the immediate and long-term effects of gamma irradiation of breast cells that may be the key to further understanding of the mechanism of radiation-induced breast cancer.

AACR Special Conference: Advances in Breast Cancer Research Abstract

Breast cancer may be induced with relatively high frequency by radiation. Ionizing radiation is one of the main treatment modalities used in the management of cancer. A radiation dose-related increase in the incidence of breast cancer has been seen in women. When a woman receives significant radiation prior to the age of 20, she becomes more likely to develop breast cancer. Thus there is considerable interest in understanding the cellular response to DNA-damaging agents, particularly because the ability to deliver a curative dose of radiation is frequently limited by the adverse reaction of normal tissues within the radiation treatment field. One approach to this problem is to understand the molecular mechanisms underlying the radiation responses of normal tissue so that critical molecular pathways can be manipulated to improve the therapeutic ratio and hence, the chance of a cure. We propose that the expression of several genes is directly affected by gamma radiation. Abnormal expression of these genes may be one of the early steps in breast carcinogenesis induced by radiation. We plan to screen breast cells that contain a single gene mutation using a technique called gene trapping. We will be able to detect changes in the expression of a specific gene upon treatment with different doses of radiation. These radiation response genes will be identified through the rapid amplification of cDNA ends (RACE) procedure and sequenced. Cells that are affected by radiation will be isolated and further analyzed to see if the changes can lead to the malignant transformation of the normal breast epithelial cell into a neoplastic cell. This assay may prove to be a powerful tool in the identification of novel genes that are affected by gamma irradiation in the early stages of breast cancer progression. This study will provide new information on the effects of radiation and genes that can cause breast cancer that are induced by radiation as well as identify markers for early detection of breast cancer and targets for therapeutic intervention.

Novel radiation response genes identified in MCF10A gene-trapped cells.

Jennifer Malone and Robert Ullrich
Department of Environmental and Radiological Health Sciences, Colorado State University
Graduate Student, 491-7497, Jennifer.Malone@ColoState.EDU

Objective/Hypothesis: In this study, we have established an assay to identify novel genes that are affected by gamma irradiation and to characterize their function and role in early breast carcinogenesis. We hypothesize that the mutation of these genes or their abnormal expression in response to gamma irradiation is one of the causes of breast carcinogenesis.

Specific Aims: The specific aims of this study are:

1. To establish a high throughput assay for detection of variation in gene expression in human mammary epithelial cells using gene-trapped MCF10A clones; 2. To determine the effect of gamma irradiation on the expression of the reporter, green fluorescent protein (GFP); 3. To characterize the effect of gamma irradiation on the transformation of human mammary epithelial cells; 4. To identify the trapped genes affected by gamma irradiation in breast epithelial cells.

Methods: We plan to establish an assay that will allow us to screen for breast cells that contain a single gene mutation using a technique called gene trapping. This will allow us to detect changes in the expression of a specific gene upon treatment with different doses of radiation. These radiation-responsive genes will be identified through the rapid amplification of cDNA ends (RACE) procedure and sequenced. Gene-trapped clones that are affected by radiation will be isolated and further analyzed by real-time PCR and compared to the parental to verify that a radiation response is being seen.

Results: The MCF10A gene-trapped library has been established and basal GFP levels have been measured. Gamma irradiation of the single cell gene-trapped clones at both 1.0 and 2.0 Gy has been performed. Clones that were either up- or down-regulated at least 2-fold in response to the radiation treatment have been expanded and analyzed by 3' RACE and sequencing. The five radiation response genes identified have been analyzed by real time PCR and cell cycle analysis.

Study Design: Using the poly-A trap retrovirus RET, we have established a gene-trapped library of clones from human mammary epithelial cells (MCF10A). This provides a strong base for the identification of novel genes that may be involved in essential signaling pathways in human mammary epithelial cells. The reporter gene GFP, which has been incorporated into the genome of the cells, monitors the expression level of the endogenous trapped genes. We will compare basal GFP expression before and after exposure to varying low doses of gamma radiation (0-4 Gy) using replica plates of MCF10A gene-trapped clones. We will then identify the genes involved by using 3' RACE and sequencing. The identified radiation response gene's mRNA levels will be analyzed by real-time PCR analysis and compared to the parental MCF10A cell line after varying doses and time points following ionizing radiation.

Conclusions: This assay may prove to be a powerful tool in the identification of novel genes that are affected by gamma irradiation in the early stages of breast cancer progression. This study will provide new information on the effects of radiation-responsive genes that can lead to breast cancer as well as identify new markers for early detection of breast cancer. This study will focus on the identification of novel genes that are potential targets of gamma irradiation. It will provide essential information on the immediate and long-term effects of gamma irradiation of breast cells that may be the key to further understanding of the mechanism of radiation-induced breast cancer.

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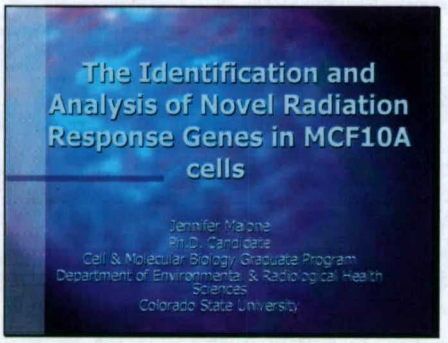

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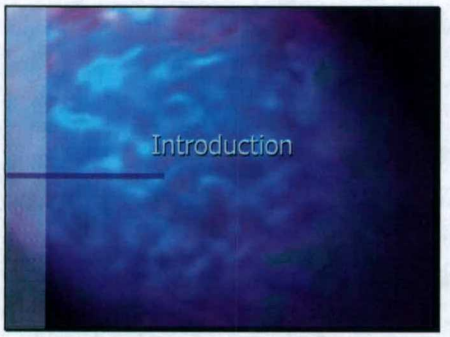

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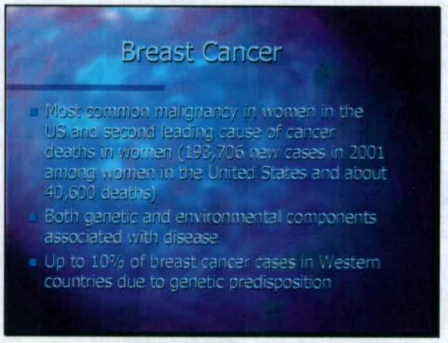

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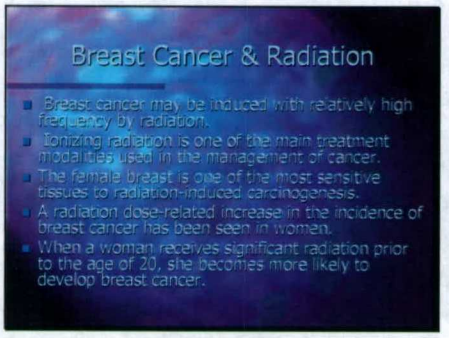

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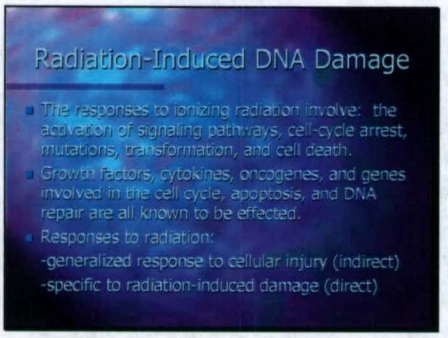

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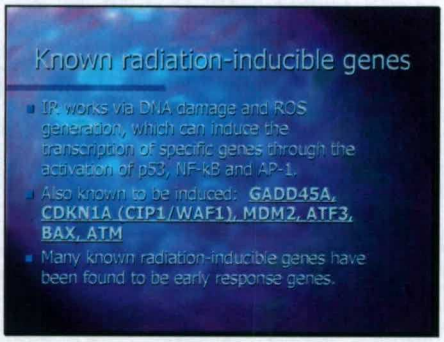

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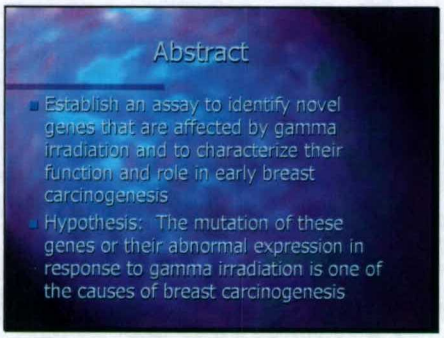


Slide 10

Study Design

- Using a poly-A trap retrovirus vector RET, a gene-trapped library of clones from MCF10A cells has been established
- Establish a detection assay using reporter gene GFP that is incorporated into the genome of the cells, whose expression is regulated by endogenous promoters of the trapped genes
- Compare basal GFP expression before & after exposure to varying low dose gamma irradiation using replica plates
- Identify genes by PCR and sequencing
- Characterize clones affected by IR by real-time PCR analysis

Slide 11

What is Gene Trapping?

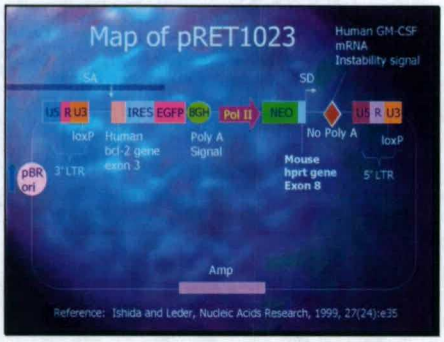
- A method of random insertional mutagenesis that uses a fragment of DNA coding for a reporter or selectable marker gene as a mutagen
- A DNA vector can be introduced into cells by transfection or infection and be randomly integrated into the genome in regions where functional genes are found
- The sequence of the 'trapped' gene can be identified using techniques that are based on the polymerase chain reaction (PCR), and this can lead to the isolation of novel genes regardless of their level of expression in vivo

Slide 12

Removable Exon Trap (RET)

- A poly-A trap retrovirus vector constructed by Ishida and Leder uses a combination of a very strong splice acceptor, an effective polyadenylation signal and a promoterless green fluorescent protein cDNA that allows the expression pattern of the trapped gene to be monitored in living cells
- The integrated provirus can be removed from the genome of infected cells by excision using homologous recombination

Slide 13



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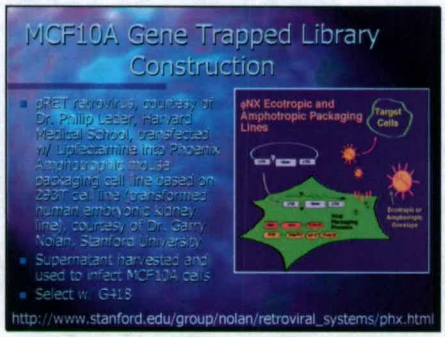

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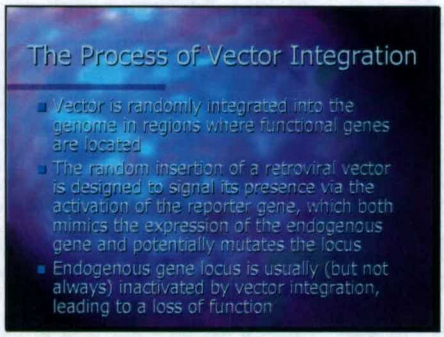


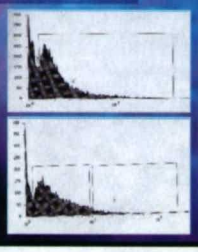
Slide 16

Cell Sorting of Infected pool of GFP clones

- Cells sorted by flow cytometry
- MCF10A gene-trapped clones were sorted by GFP expression into **positive** and **negative** pools
- GFP positive pool was further sorted into high, medium, and low expression levels

Slide 17

Flow Cytometry Analysis



Initial sort of gene-trapped MCF-10A clones into GFP+ and GFP- pools. Sort gate 1 is the GFP- pool and sort gate 2 is the GFP+ pool.

Cell sort of gene-trapped MCF-10A clones into high, medium and low GFP expression levels. Sort gate 1 is the GFP medium pool and sort gate 2 is the GFP high pool.

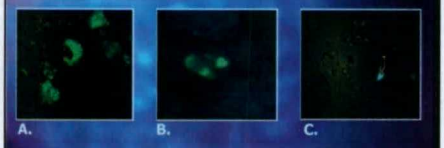
Slide 18

Replica Plating

- Pooled clones of gene-trapped MCF10A clones plated by limiting dilution
- (1 cell/well) in 96-well plates
- Expand in presence of G418
- Once confluent, replica plating was done; 6-96 well plates per each GFP- and GFP+ sorted clonal cell population
- The 6 plates were for: basal GFP detection, master, store at – 80 degrees, 2.0 Gy , 1.0 Gy, and control

Slide 19

GFP expression of replica plated gene-trapped MCF-10A clones



A. High GFP expressing M1023 gene-trapped clone from replica plate
B. Medium GFP expressing M1023 gene-trapped clone from replica plate
C. Low GFP expressing M1023 clone from replica plate

Slide 20

Detection of basal GFP expression using microplate reader

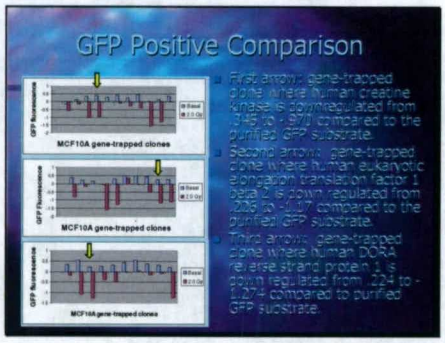
- BioRad microplate reader was used to quantitate GFP expression by a sandwich ELISA assay
- GFP detection in the 96-well plates was performed by scanning at 450 nm
- GFP absorbance was quantitated using Microplate manager software
- Readings taken both before and after ionizing radiation treatment

Slide 21

Exposure to gamma irradiation

- MCF10A clones were subjected to a 2.0 Gy dose of gamma irradiation by a sealed ^{137}Cs source
- Gamma irradiation is a known mutagenic agent
- GFP expression quantified with microplate reader immediately following radiation exposure

Slide 22



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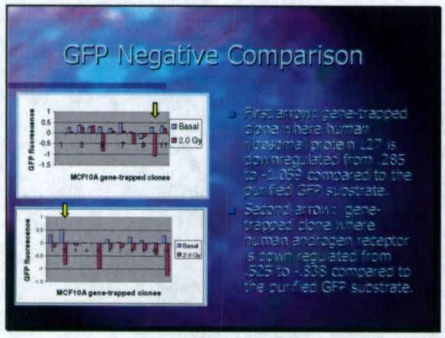

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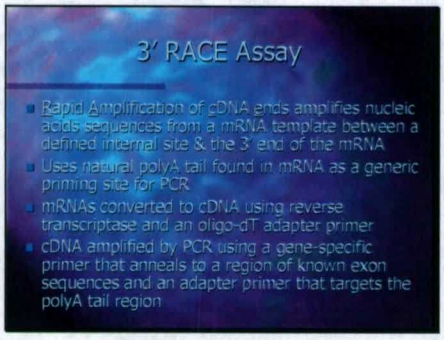


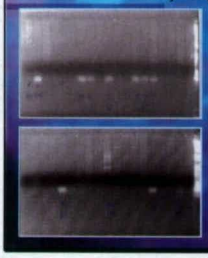
Slide 25

3′ RACE continued

- Permits capture of unknown 3′ mRNA sequences that lie between the exon and polyA tail
- PCR products cloned into sequencing vector using Zero Blunt TOPO Cloning kit from Invitrogen
- Nucleotide sequence of purified PCR fragments determined by sequencing with the M13 universal primers
- Search BLASTX & BLASTN databases for homologous genes

Slide 26

Gel Electrophoresis: PCR of Miniprep DNA



- The bands seen in the agarose gels to the left correspond to the PCR amplified region between the 5D site and the NEO cassette, which is roughly around 800 bp
- A 1 Kb Plus DNA ladder is used as a reference
- The clones pictured here, along with others for a total of 31 clones, were sequenced using M13 universal primers at Davis Sequencing at UC Davis

Slide 27

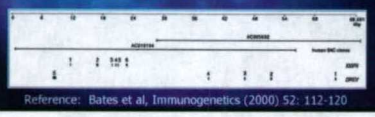
Sequencing Results

- 31 irradiated gene-trapped clones sequenced
- Sequencing results plugged into BLAST
- 5 clones were homologous to known gene sequences:
  - Human DORA reverse strand protein 1 (DREV1)
  - Human Androgen Receptor
  - Human Eukaryotic Translation Elongation Factor 1 Beta 2 (EEF1B2)
  - Human Creatine Kinase Gene
  - Human Ribosomal Protein L27

Slide 28

Human DORA reverse strand protein 1 (DREV1)

- The small gene DORA is embedded in intron 4 on the complement strand
- Has homology to DNA methyltransferases
- Recognize specific DNA sequences and transfers a -CH_3 from the cofactor SAM to 5' position of cytosine included in CpG dinucleotides.
- Most of them contain Sp1 elements whose removal may induce methylation of the associated island.

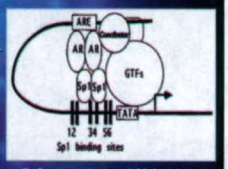


Reference: Bates et al, Immunogenetics (2000) 52: 112-120

Slide 29

Androgen Receptor (AR)

- ↑p21 (WAF1, CIP1, SDI1, CAP20) gene
- AR and transcription factor Sp1 interact
- Regulates several cell cycle-regulatory molecules such as CDK2 & 4 that promote cell proliferation
- Inhibits p16 family of CDK inhibitors
- Interacts with Prostate-specific antigen (PSA) and members of the Kallikrein gene family



Reference: Lu et al Molecular Epidemiology 14(5):753-760 2000

Slide 30

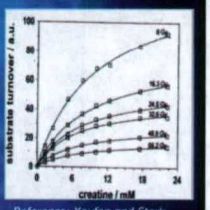
Human Eukaryotic Translation Elongation Factor 1 Beta 2

- Located on chromosome 2
- Expressed in a wide range of tissue types, expected of a single gene encoding protein predicted to be essential
- rare, recessive, juvenile-onset motor neuron disease/amyotrophic lateral sclerosis (ALS2) mapped to this region
- Elongation factors may constitute up to 5% of the total cellular protein in actively proliferating cells--tumor & cultured cells express levels up to 20-fold higher than normal

Slide 31

Human Creatine Kinase (CK)

- Inactivated by free radical damage – indirect radiation effects
- Inactivation of CK by ROS might be involved in energy metabolic regulation in cancer
- CK activity lower in cancer tissues than in normal tissues
- E6 protein from papilloma virus promotes degradation of p53 resulting in increased CK.



Reference: Koufen and Stark, Biochimica et Biophysica Acta 1501 2000, 44-50

Slide 32

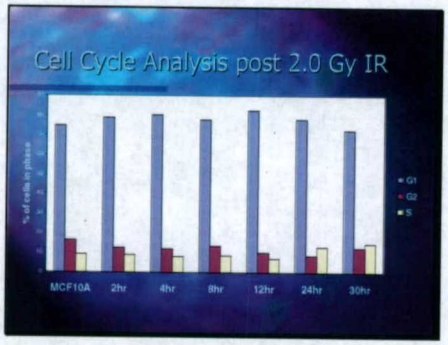
Human Ribosomal Protein L27

- Ribosome responsible for protein synthesis
- Located on chromosome 17
- Evidence has accumulated indicating that a number of ribosomal proteins have a second function apart from their role in the ribosome and in protein synthesis

Slide 33

Results

Slide 34



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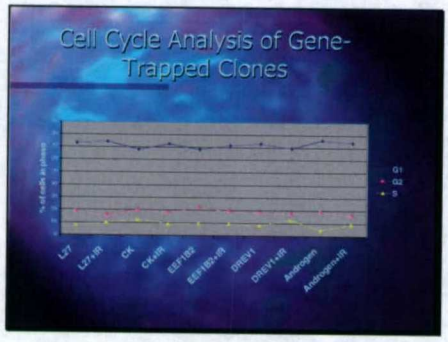

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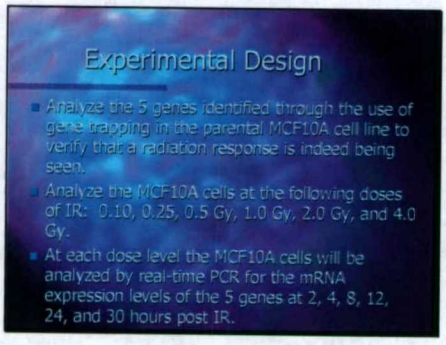


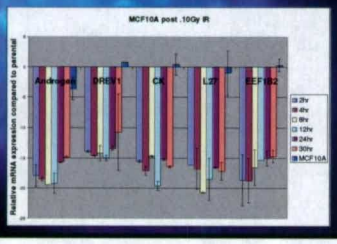
Slide 37

Data Calculations

- Absolute Standard Method for data quantification:
  ΔCT = CT (treated samples) - CT (MCF10A)
- Relative gene expression level for data quantification:
  $= 2^{-\Delta\Delta CT}$ **(Reference: Methods 25, 402-408 2001)**
  **Data expressed as the fold change in gene expression normalized to an endogenous reference gene and relative to the untreated control.
  **Untreated control sample $\Delta\Delta CT = 0$ & $2^0 = 1$, so fold change in gene expression relative to the untreated control = 1.
- Assumptions of this method: amplification efficiencies of target & reference must be approximately equal. If not equal you need to use the absolute quantification method using standard curves.
- Internal control housekeeping gene (GAPDH) and untreated control calibrator (MCF10A) are needed for the $2^{-\Delta\Delta CT}$ method.

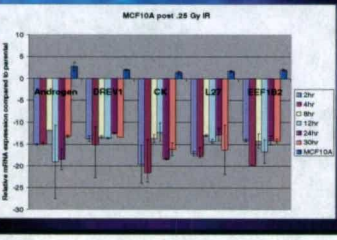
Slide 38

mRNA expression level at 0.10 Gy relative to parental



Slide 39

mRNA expression level at 0.25 Gy relative to parental



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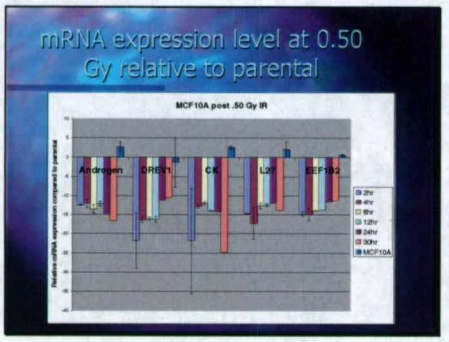

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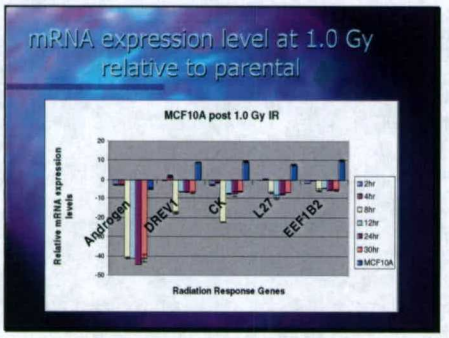

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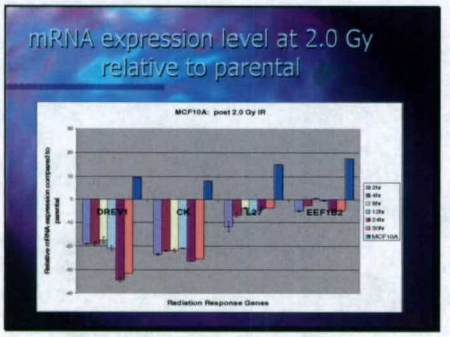

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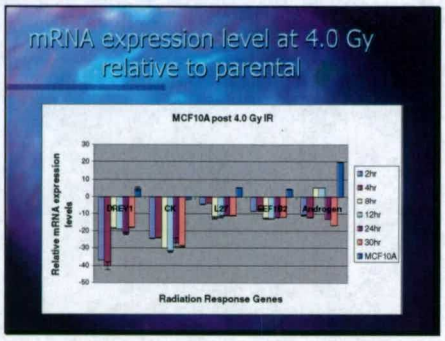
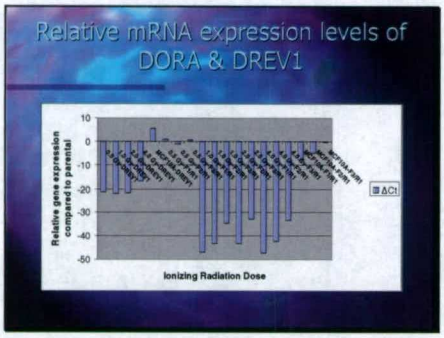


Slide 44



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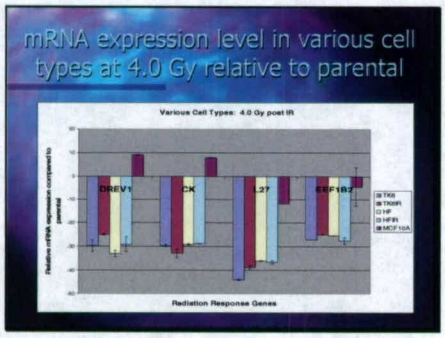


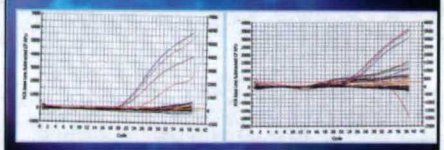
Slide 46

RNAi

- siRNAs will be targeted to the DREV1 gene in the MCF10A cells
- mRNA expression levels of DREV1 analyzed by real-time PCR
- Cells scored for chromosomal & chromatid aberrations

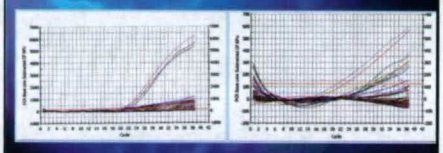
Slide 47

Real-time analysis of DREV1 siRNA knockdown



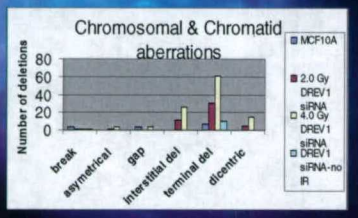
Slide 48

Real-time PCR analysis of DREV1 siRNA knockdown



Slide 49

Analysis of chromosomal & chromatid aberrations



Slide 50

Conclusions

- Five novel, previously unidentified radiation responsive genes found
- All five genes are significantly downregulated relative to the parental following IR treatment at the following doses: 0.10, 0.25, 0.50, 1.0, 2.0, and 4.0 Gy
- Radiation response seen is not cell-type specific
- Knockdown of DREV1 gene expression does not appear to affect cell survival

Slide 51

Acknowledgements

- Dr. Robert Ullrich
- Members of the Ullrich Lab
- Committee Members:

  Dr. Sue Lana, Dr. Bill Hanneman, & Dr. Mike Fox

My family for their support throughout this process.

NOVEL RADIATION RESPONSE GENES IDENTIFIED IN MCF10A CELLS

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Introduction

Breast cancer may be induced with relatively high frequency by radiation. Ionizing radiation is one of the main treatment modalities used in the management of cancer. Radiation is used in the treatment of breast cancer, leukemia, and Hodgkin's lymphoma to kill cancerous cells. While the use of medical radiation has undoubtedly prolonged and saved the lives of many, it is not without side effects. A radiation dose-related increase in the incidence of breast cancer has been seen in women. When a woman receives significant radiation prior to the age of 20, she becomes more likely to develop breast cancer. Thus there is considerable interest in understanding the cellular response to DNA-damaging agents, particularly because the ability to deliver a curative dose of radiation is frequently limited by the adverse reaction of normal tissues within the radiation treatment field. One approach to this problem is to understand the molecular mechanisms underlying the radiation responses of normal tissue so that critical molecular pathways can be manipulated to improve the therapeutic ratio and hence, the chance of a cure.

We propose that the expression of several unknown genes is directly affected by gamma radiation. Abnormal expression of these genes may be one of the early steps in breast carcinogenesis induced by radiation. We have established an assay that allows us to screen for breast cells that contain a single gene mutation using a technique called gene trapping. Gene trapping is a form of insertional mutagenesis specifically designed to disrupt gene function by producing intragenic integration events. By employing a polyadenylation (poly A) trap a mRNA transcribed from a selectable marker gene lacking a poly A signal in a gene-trap vector is stabilized only when a gene-trap vector captures a poly A signal. Since poly A trapping occurs independently of the expression of the target genes, any gene could potentially be identified at almost equal probability regardless of the relative abundance of its transcripts in target cells. Upon treatment with ionizing radiation, the radiation-response genes identified will be sequenced through the rapid amplification of cDNA ends (RACE) procedure.

Hypothesis and Rationale

Mutation of novel genes or their abnormal expression in response to a single dose of gamma radiation is one of the causes of early breast carcinogenesis.

In experimental models, ionizing radiation induces mammary transformation both *in vivo* and *in vitro*, however, the cellular and molecular mechanisms of radiation-induced carcinogenesis are not known. To understand the mechanisms, it is necessary to determine the conditions that modulate the susceptibility of this target tissue to ionizing radiation. The assessment of the transforming ability of ionizing radiation on breast epithelium requires a highly proliferating cell population, which in turn has to express normal breast phenotypes. Since ionizing radiation induces features of neoplastic transformation in human breast cells, the identification of malignant phenotypes involved in breast cancer are of critical importance in understanding the pathogenesis of the disease.

The aim of our assay is to identify genes responsive to gamma irradiation through the use of gene trapping. Gene trapping will tag the radiation-responsive genes and we will be able to monitor their expression levels using GFP. At the same time, we will be introducing a single allele gene disruption into the radiation-responsive gene by the integration of our poly A trap vector which will decrease expression. We believe that by utilizing the gene-trapping technique some of the unknown genes that are contributing to familial and non-familial breast cancer can be identified. During radiation treatment of breast cancer, some of the cells surrounding a targeted tumor also become exposed, leading to mutations. It is of great importance that these radiation-induced mutations be identified so that a secondary cancer does not develop. The radiation-responsive genes identified can then serve as markers for screening and hopefully aid in early detection.

Methods

MCF-10A is a spontaneously non-tumorigenic immortalized human breast epithelial cell line. We have generated RET-infected G418-resistant MCF-10A clones, which essentially are a gene-trapped library of mammary epithelial cells. This library can be used in identifying genes that are activated or inhibited in mammary epithelial cells in response to different genotoxic agents or developmental signals. Since the infection rate is one virus per cell, this library represents cells in which one functional gene is disrupted by the integration of the vector. It provides the strong base for the identification of novel genes that may be involved in essential signaling pathways in human mammary epithelial cells.

We have established a detection assay using the reporter, Green Fluorescent Protein (GFP), that has been incorporated into the genome of the clones and whose expression is regulated by the endogenous promoters of the trapped genes. We will compare basal GFP expression before and after exposure to varying low dose gamma radiation (0-4 Gy) using replica plates of MCF10A clones. We will then identify the gene/s involved by using a polymerase chain reaction protocol (3' RACE) and sequencing analysis. Next, we will further characterize the clones that are affected by gamma irradiation by performing real-time PCR to analyze gene expression, soft agar assays to analyze anchorage-independent growth and tumorigenicity assays to confirm if the gene trap clones cause transformation.

Results

The gene-trapped MCF-10A clones were sorted by flow cytometry into GFP+ and GFP- expression levels. The GFP+ sort pool was then further sorted into GFP high, medium and low expression levels. Replica plates were made for both the GFP+ and GFP- sorts. The GFP expression level was measured using an ELISA assay both before and after a 2.0 Gy dose of IR.

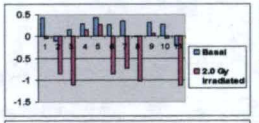

Figure 1. GFP negative sort population of MCF10A gene trapped single cell assay clones. A small minority of clones were upregulated, but most of the clones were downregulated.

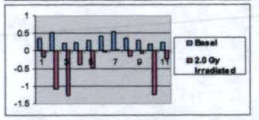

Figure 2. GFP positive sort population of MCF10A gene trapped single cell assay clones. Clones that were either up or down-regulated at least 2-fold in comparison to basal levels were expanded to determine what gene was trapped.

3' RACE analysis was implemented to determine the identity of the trapped genes in the MCF10A clones that were up- or down-regulated in response to a 2.0 Gy dose of ionizing radiation. The five genes identified through a BLAST homology search were:

Human DORA reverse strand protein 1 (DREV1) : **G10+**
Human Androgen Receptor : **G11-**
Human Eukaryotic Translation Elongation Factor 1 Beta 2 : **E8+**
Human Creatine Kinase Gene : **B5+**
Human Ribosomal Protein L27 : **A4-**

Quantitative real-time PCR analysis of mRNA expression of gene-trapped MCF10A clones irradiated with 2.0 Gy ionizing radiation and MCF10A epithelial cells harvested at various time points after 2.0 Gy dose. The following calculation was done to obtain the results presented in the graphs below:

$2^{-\Delta\Delta Ct}$

This formula calculates the relative gene expression by using the Ct values obtained from a PCR base line subtracted graph calculated by the iCycler software. The Ct values from the MCF10A gene trapped clones were compared to the endogenous control GAPDH to obtain the ΔΔCt values. MCF10A parental cells were used as a calibrator and set to 1.

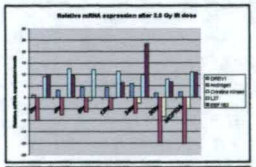

Figure 3. Real-Time PCR analysis of MCF10A gene-trapped IR clones. The relative mRNA expression levels of the IR gene-trapped clones to the parental MCF10A cell line at various time points following IR.

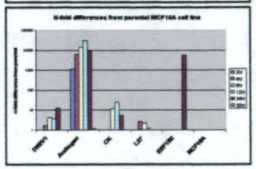

Figure 4. Real-Time PCR analysis of MCF10A gene-Trapped IR clones. The fold change in mRNA expression level of the IR gene-trapped clones compared to the parental line, MCF10A.

Cell cycle analysis was performed by flow cytometry to investigate if the DNA content varied during the phases of the cell cycle after a 2.0 Gy dose of ionizing radiation. Analysis was performed on the parental MCF10A cell line without IR and at varying time points after a 2.0 Gy dose of IR. This analysis was also performed on the five gene-trapped clones both with and without a 2.0 Gy dose of IR. This experiment was done to disregard any fluctuations in the mRNA expression levels being due to a cell cycle phenomenon.

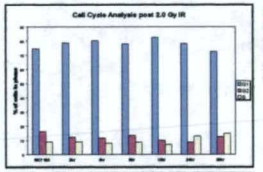

Figure 5. Cell cycle analysis of DNA content post IR. Analysis was performed after a 2.0 Gy dose of IR and analyzed at various time points post IR via flow cytometry. MCF10A parental cell line was used as a control.

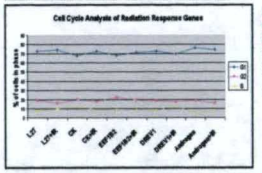

Figure 6. Cell cycle analysis of DNA content in potential radiation response gene-trapped clones. The DNA content in G1, G2, and S-phase was performed by flow cytometry analysis. The gene-trapped clones were analyzed with and without a 2.0 Gy dose of IR.

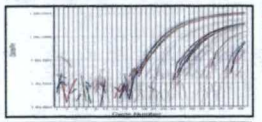

Figure 7. Amplification plot: Delta Rn vs. Cycle number. Real-time PCR experiment analyzing the mRNA expression levels of the five radiation response genes at various doses of IR.

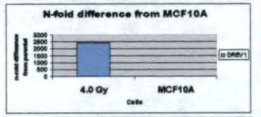

Figure 8. Real-Time PCR analysis of DREV1 mRNA expression. The N-fold difference in mRNA expression levels of DREV1 in MCF10A cells with either a dose of 4.0 Gy or without IR.

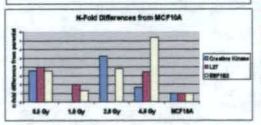

Figure 9. Real-Time PCR analysis of mRNA expression levels. The N-fold difference in mRNA expression levels of MCF10A at various doses of IR for the following genes: creatine kinase, ribosomal protein L27, and translation elongation factor 1 beta 2.

Conclusions

This study identified five genes that are potential targets of gamma irradiation. The immediate and long-term effects of gamma irradiation on breast cells may be the key to further understanding the mechanism of radiation-induced breast cancer. This assay will also be useful for testing other potential environmental risk factors involved in breast carcinogenesis that may prove to be useful as markers for early detection of breast cancer and targets for therapeutic intervention.

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